C·R·E·S®
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

REPORT
WINTER 1998

The Frozen Zoo: Extending the Living Animal Collection

The Frozen Zoo is alive and well, although not bleating or blooming in the same manner as its animal and plant collection counterparts. Unseen by the public, the Frozen Zoo is maintained by the Zoological Society of San Diego as part of the genetics program at CRES, where the concept of living collections has been extended to include one of the largest inventories of living cells in the world.

Housed in an array of large cryogenic freezers, the Frozen Zoo contains carefully accessioned and cryopreserved cells, representing a wide range of mammals and birds. It is composed of two parts: somatic cells (diploid fibroblast skin strains) and gametes (haploid sperm and eggs). The gamete collection, overseen by the reproductive physiology division, includes semen samples from approximately 750 animals and eggs from another 75 individuals. The collection of somatic cells, however, comprises the bulk of the Frozen Zoo, with cells from 3,679 individual mammals and 26 birds.

Begun more than 20 years ago, the Frozen Zoo started as a resource for in-house research endeavors and as a way of preserving the genomes of animals for future studies. Today it is an incredibly valuable resource not only for in-house research but also for scientists around the world who are interested in studying the genomes of threatened and endangered species. Where else can they get cells of an elephant, a giant panda, or a gorilla?

From small ear notch or skin biopsy samples collected opportunistically during routine veterinary examinations or at necropsy, cell cultures are established and grown until multiple vials of each individual can be frozen. Cells immersed in a special cryoprotectant medium are frozen at a rate of 1 degree Centigrade per minute and then placed in liquid nitrogen holding tanks for storage at -196 degrees Centigrade (-385 degrees Fahrenheit). Samples are split between freezers in two different locations to ensure safekeeping of the cells.

Continued on page 2

Without captive breeding efforts such as those under way at the Wild Animal Park, the Somali wild ass faces possible extinction. Through the Frozen Zoo, cytogenetic studies focus on identifying chromosomal differences within captive populations.

CRES® is operated by the Zoological Society of San Diego.

Cheetah Reproduction
Hormone studies under way at the Wild Animal Park. See page 3.

Adopt-An-Animal for the Holidays!
Gorillas are the focus for DNA studies at CRES. See page 5.

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The Frozen Zoo: Extending the Living Animal Collection

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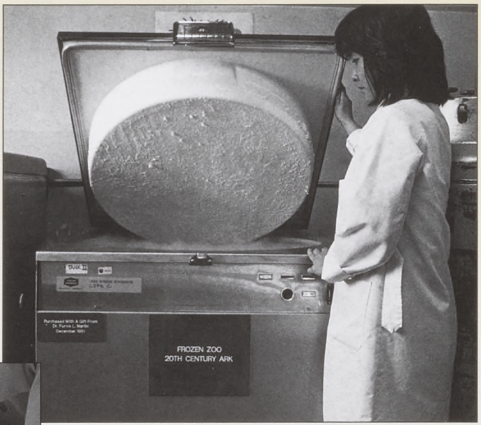
The acquisition priorities have been centered on mammalian species, and emphasis is on the orders Artiodactyla (even-toed ungulates), Perissodactyla (odd-toed ungulates), Primates, and Carnivora, with 13 of the 26 mammalian orders represented. Approximately half of the species are on protection lists of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), an international treaty that protects animals and plants from illegal importation and exportation. Also targeted are samples from species that are part of the American Zoo and Aquarium's (AZA) Species Survival Plan (SSP) program. Species that are designated as an SSP program are managed collectively among zoos in intensive programs using rigorous genetic and demographic analyses as the basis of management. Of the 56 mammal SSP programs, 46 are represented in the frozen collection at CRES (see Table 1).

Samples from the Frozen Zoo have been used in-house at CRES for virtually all of the genetic studies conducted by the cytogenetics and the molecular genetics divisions. They have been used in studying genetic variation within and between populations of animals such as the gorilla, rhinoceros, bonobo, and dik-dik. Cytogenetic studies have focused on identifying chromosomal differences within captive populations, as in the Somali wild ass, suni antelope, and waterbuck populations. In cases of unknown parentage, cryopreserved cells have been useful in genetic evaluations to determine paternity or maternity. Frozen cells are particularly valuable in retrospective studies: an animal may have died, but there are still living cells from that individual in the Frozen Zoo that can be thawed for study in the future. There may be genetic, disease, or other medical questions that arise in the future that may depend on frozen cells for answers.



Several large cryogenic freezers like this one constitute the Frozen Zoo, seen here with author Arlene Kumamoto. Its contents of cells and gametes have been called the 20th Century Ark.

With the arrival and growth of genetic technologies, this collection of cells has come to the attention of scientists around the world who are interested in studying issues of biodiversity, genetics, and systematics. In addition, the Human Genome project, the largest effort to date to define the entire human genome, has sparked interest in other species that can help researchers understand and elucidate the human genetic material. Samples from animal species are of tremendous help to these researchers. The scientific world was startled in 1997, when researchers in Scotland took a nucleus from cells like those in the Frozen Zoo and transferred it into another enucleated somatic cell. The result was Dolly, a lamb that was a clone of its mother. It is because of scientific advances such as this that we cannot foresee all the potential uses of this precious repository.

Table 1–Species Represented in Frozen Zoo

Artiodactyls	
Addax	*(15)*
Babirusa	*(2)*
Barasingha	*(13)*
Chacoan Peccary	*(11)*
Gaur	*(5)*
Okapi	*(54)*
Oryx, Arabian	*(44)*
Oryx, Scimitar-horned	*(47)*
Carnivores	
Bear, Sloth	*(9)*
Bear, Spectacled	*(5)*
Bear, Sun	*(6)*
Black-footed Ferret	*(2)*
Cheetah	*(17)*
Giant Panda	*(2)*
Leopard, Clouded	*(8)*
Leopard, Snow	*(5)*
Lion	*(5)*
Maned Wolf	*(1)*
Red Panda	*(12)*
Small-clawed Otter	*(9)*
Sumatran Tiger	*(8)*
Proboscideans	
Elephant, African	*(3)*
Elephant, Asian	*(5)*
Perissodactyls	
Asian Wild Horse	*(395)*
Rhino, Black	*(68)*
Rhino, Indian	*(9)*
Rhino, Sumatran	*(8)*
Rhino, White	*(42)*
Zebra, Grevy's	*(35)*
Zebra, Hartmann's Mt.	*(113)*
Primates	
Bonobo	*(50)*
Chimpanzee	*(15)*
Colobus	*(26)*
Drill	*(20)*
Francois' Monkey	*(24)*
Gibbons	*(10)*
Goeldi's Monkey	*(5)*
Gorilla	*(128)*
Lemur, Black	*(4)*
Lemur, Ring-tailed	*(5)*
Lemur, Ruffed	*(32)*
Lion-tailed Macaque	*(45)*
Mangabey	*(8)*
Orangutan	*(20)*
Pygmy Loris	*(12)*
Marsupials	
Tree Kangaroo	*(14)*

Scientific advances and increased interest in the Frozen Zoo as a biological resource for research investigations have encouraged the CRES genetics division to expand the Frozen Zoo even further. Plans are under way to work with field biologists to increase the number of species represented: samples will come not only from far-off places but also from our own backyard. Samples from local fauna, as well as from neighboring Baja California, will help build the collection so that studies can be undertaken on local species diversity. Techniques are currently being developed to culture and freeze cells from birds by using the minute bits of cellular material at the end of bird feather shafts. Reptile and amphibian cells are also on the horizon for the Frozen Zoo. With these additions, the Frozen Zoo may truly become the 21st Century Ark.

—Arlene Kumamoto
Associate Researcher
Cytogenetics/CRES

INSIDE CRES

Reproductive Physiology

The San Diego Wild Animal Park's cheetah breeding facility, under the direction of CRES researchers, has produced more than 100 cheetah cubs during the 1980s and early 1990s. In fact, reproduction was so good that a pause in breeding acitivity was suggested by the cheetah Species Survival Plan. Several years later, we have received the green light to begin breeding once again. However, many of the females in our group are now approaching the end of their reproductive life span and are not cycling regularly. The CRES reproductive physiology and behavior divisions have developed a protocol for stimulating estrus and ovulation in this group of reluctant females. First, each female's reproductive tract is examined by both ultrasound and direct observation via laparoscopy. A hormone treatment is designed based on the status of follicle (the structure containing the ovum) growth in the ovaries.The hormones mimic the natural cycle of the female, stimulating follicle growth and ovulation.This treatment has been used four times with three cheetahs, resulting in estrus and breeding in three cases, as well as resulting in at least one pregnancy. As we learn more about the cheetah's ovarian response to hormone therapy, we will further refine the protocol. We are confident that we will soon be hearing the pitter-patter of little cheetah paws once again at the Wild Animal Park.

Grants

The Center for Reproduction of Endangered Species is grateful to the following for their support of imperiled wildlife:

The **Heller Foundation of San Diego** has awarded a Bud Heller Conservation Fellowship to the Zoological Society for a behavior and endocrinology study of the endangered bonobo, or pygmy chimpanzee. It is anticipated that the results of this study will lead to a greater understanding of reproduction of this vanishing species. **The Amerman Family Foundation** has made a grant in support of the California Condor Recovery Program to monitor parental care behavior for the 1998-1999 breeding and hatching season. Data acquired from this study will benefit scientists in the wild at release sites as well as those dedicated to the captive collections. **The Muchnic Foundation** has given a grant to the CRES endowment to ensure the continuation of conservation research.

Cans for Critters is 19 Years Old in 1999!

It took one famous place-kicker, one dedicated teacher, and one Del Mar school to start what has become an award-winning, conservation-driven recycling program for school-aged children throughout southern California.

In its 18 years, Cans for Critters has donated more than $275,000 to support CRES, which is dedicated to developing, gathering, and increasing knowledge that is vital for the establishment of self-sustaining wildlife populations.

Do your part to continue the CRES commitment to research and conservation by participating in the Cans for Critters recycling program. To join the program, learn drive dates, and receive incentives for yourself, your school, troop, or company, please call Kathleen Rafaat at (619)557-3995 for more information. Or just save your aluminum cans, take them to your favorite recycler, and send your check made out to CRES to: Zoological Society of San Diego, P.O. Box 120551, San Diego, CA 92112, Attention: Cans for Critters.

1998 Bonner Summer Research Fellows

This is the fifth year that young scientists have benefited from the Bonner Summer College Student Fellowship Endowment Fund, thanks to the generosity of Jewell H. and James T. Bonner. Since 1994, two students have been selected each year as summer interns to work on research projects at CRES before they continue with their graduate studies. You can be a part of these endeavors by helping to sponsor one of our CRES summer interns. For more information, please call Amy Parrott in the San Diego Zoo's Development Office at (619) 685-3213.

Inger Britt Carlsson (left) and Helene Jensen studied giant panda fertility and reproduction from different perspectives.

This past summer, I had the opportunity to work for the San Diego Zoo: not only did I work for the Zoo, but I also saw the work going on behind the scenes at CRES. When I heard that a Bonner Fellowship was being offered by the analytical chemistry division of CRES, I knew this was a chance to put my chemistry skills to work on behalf of endangered species.

Working under the guidance of Dr. Lee Hagey at CRES, I experienced several different areas of interest. These included the historical evolution of animals through the study of bile acids; the elemental composition of rhinoceros blood; analysis of scent samples; and the summer's major project, determining if the female giant panda, Bai Yun, was pregnant as a result of artificial insemination. This year there was a new approach taken in monitoring Bai Yun's hormonal levels: her steroid levels were monitored from just a single drop of urine.

I was able to run the instruments, search for unknown steroids, and compare the many samples taken from Bai Yun with samples taken from other pregnant and nonpregnant giant pandas in China. Although there was no pregnancy, the information collected will be valuable for future indicators of pregnancy for Bai Yun.

My research also involved analysis of communication between animals by studying their scent marks, or pheromones. Using the instruments in the analytical chemistry laboratory, I identified the different chemical compounds that make up scent marks. It is amazing how something that is invisible and odorless to humans can contain so many chemical messages for other animal species.

As a Bonner Fellow at CRES, I learned about the important relationship between science and animals and how such essential studies contribute to the preservation and protection of wildlife. Now that I am back at the University of California, San Diego (UCSD), I have a renewed interest in chemistry and a focus for future studies.

—Helene Jensen

While at CRES this past summer, working under the supervision of Dr. Barbara Durrant in the reproductive physiology division, I worked on a wide variety of projects involving a diverse group of animals.

First, I collected semen samples by various methods. After the collection, samples were analyzed back at the lab, with semen quality evaluated on the basis of motility, vital stains, concentration, and morphology. After analysis, it was determined whether or not the sample would be frozen and placed in the Frozen Zoo with other tissue samples. Because there is a species-to-species variation, it is not always known which method of freezing will be best for each sample. For example, cheetah semen survives freezing best if it is cooled for 30 minutes and then frozen over liquid nitrogen vapor for 15 minutes, but Arabian oryx semen thaws better if it is cooled for 2½ hours before freezing. For this reason, we design studies to freeze a particular sample in as many as 21 different ways, and then we thaw the samples to determine the best method of freezing for that species. I have performed two large thaws to be used in determining the best method of freezing canid sperm.

Another major project involved the female giant panda, Bai Yun. Once a week, we collected vaginal swabs from Bai Yun. Analyzing vaginal cells allows us to monitor estrus in the female. As Bai Yun approached estrus, the cells changed shape and color (after PAP staining). Once it was determined that Bai Yun was ovulating—and that the male, Shi Shi, was not going to breed with her—a research team was assembled to artificially inseminate her. The semen sample was then collected from Shi Shi; it was evaluated and washed before Bai Yun was artificially inseminated.

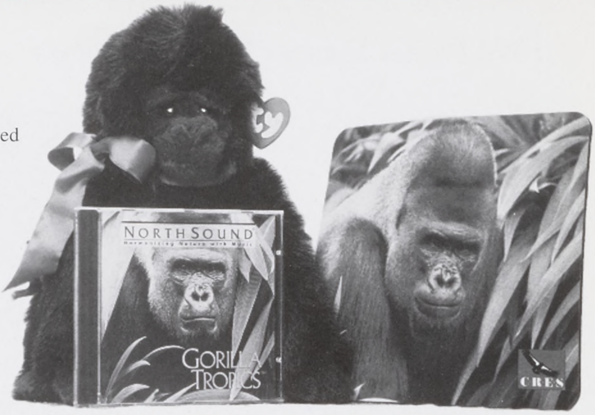
In addition, I performed a variety of other studies, which included collecting oocytes from canine ovarian tissue for growth and maturation studies; attempting semen collection on a kiwi; compiling cheetah and drill semen data for analysis; measuring and photographing mammalian reproductive tracts; and collecting data for histological analysis of ovarian follicle health and growth in the domestic dog and also in the coyote.

These and other CRES projects have reinforced my belief in conservation. Working at the CRES facility has exposed me to a wide variety of people, animals, and scientific methods, and it allowed me to work with keepers, animal trainers, and scientists from many disciplines who are invaluable contributors to these studies. I hope to continue my association with CRES as I pursue my graduate education in this field.

—Inger Britt Carlsson

Adopt a Gorilla for the Holidays!

Put some muscle into your holiday giving by sending a unique gift that also helps endangered species. Come and visit our gorilla families, both at the San Diego Zoo's Gorilla Tropics and at the Wild Animal Park, where you can see the fascinating interactions between these incredible and intelligent great apes. There are CRES research projects that directly benefit gorillas: DNA analysis to determine paternity of gorillas in zoos, which is critical for successful conservation of small populations; and DNA analysis of gorillas in the wild, with comparison of infertility rates between free-ranging mountain gorillas and captive lowland gorillas. Your contributions to the Adopt-An-Animal program benefit CRES, the Society's Center for Reproduction of Endangered Species, and its continuing efforts to learn more about endangered animals and their habitats. Adoption packages are appealing to all ages and include:



PACKAGE 1:

- 12-inch Ty, Inc. Baby George plush gorilla adorned with a holiday ribbon
- Adoption certificate
- Subscription to *CRES Report*
- Card to announce your gift

AND

- Two complimentary guest passes to the San Diego Zoo or the San Diego Wild Animal Park.

or

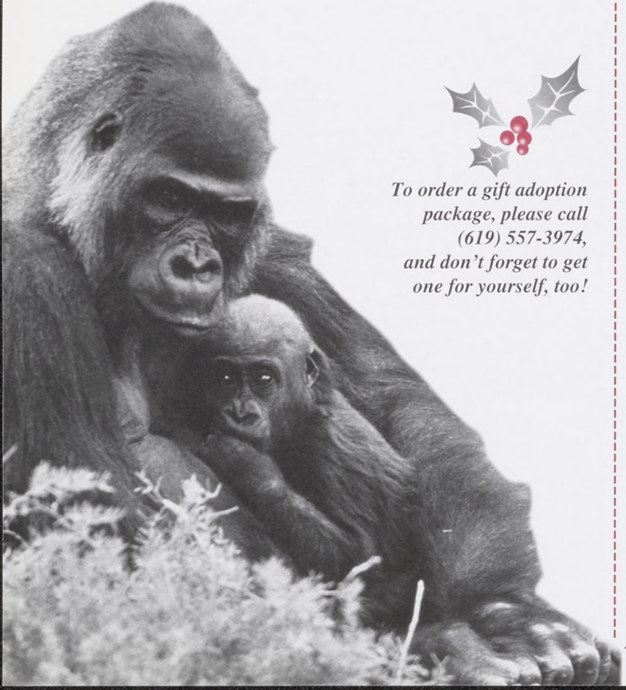
PACKAGE 2:

- 12-inch Ty, Inc. Baby George plush gorilla adorned with a holiday ribbon
- Adoption certificate
- Subscription to *CRES Report*
- Card to announce your gift

AND

- Custom computer mouse pad and a NorthSound Gorilla Tropics CD.

To order a gift adoption package, please call (619) 557-3974, and don't forget to get one for yourself, too!



ANIMAL ADOPTION APPLICATION

☐ **YES,** I want to adopt a gorilla for:

Choose: ☐ *PACKAGE 1* *or* ☐ *PACKAGE 2*

Name(s) ______________________________

Address ______________________________

City ____________________ State _____ Zip __________

Gift card message ______________________________

____

Additional gorilla adoption, *choose:* ☐ *PACKAGE 1* *or* ☐ *PACKAGE 2*

Name(s) ______________________________

Address ______________________________

City ____________________ State _____ Zip __________

Gift card message ______________________________

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Additional gorilla adoption, *choose:* ☐ *PACKAGE 1* *or* ☐ *PACKAGE 2*

Name(s) ______________________________

Address ______________________________

City ____________________ State _____ Zip __________

Gift card message ______________________________

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Please make checks payable to the Zoological Society of San Diego and mail to: The Zoological Society of San Diego, Adopt-An-Animal Program, P.O. Box 120271, San Diego, CA 92112.

OR, please charge my: ☐ MasterCard® ☐ VISA® ☐ Discover®

Card # ____________________ Exp. Date __________

Signature ______________________________

Donor Name (print) ______________________________

Daytime Phone # ______________________________

OR, you may charge your adoption by telephone: call (619) 557-3974 between 8 A.M. and 4 P.M., Monday through Friday.

Orders must reach the San Diego Zoo by December 16 to ensure delivery by Christmas.

(AHCR8)

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CRES REPORT
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CRES "Wish List"

The work is never done for CRES scientists, with all nine divisions engaged in various studies on reproduction, behavior, diseases, and habitat conservation for endangered species. There are several current projects that are in need of specialized equipment and supplies, which include:

- ★ **Digital photo document system.** Needed to photograph and analyze scientific subjects. This would be used by several CRES divisions - $4,344.
- ★ **Laminar flow hood.** Provides a sterile work space for reproductive physiology division and is needed in preparing media, culture plates, and working with ova/embryos - $6,600.
- ★ **Tabletop refrigerated centrifuge.** Needed for DNA work in genetics division, replacing outdated centrifuge that is being used by several divisions - $10,550.
- ★ **Microscope with Nimarski Optics.** Endocrinology department needs this for embryology studies - $10,000.
- ★ **Heat/air-conditioning unit for CRES primate facility.** This will be used in the keeper kitchen area. A wall unit will cool food preparation areas in the summer, keeping equipment from failing, and will help minimize floor heater safety hazards in the winter - $1,500.
- ★ **Security-controlled entry system for CRES.** Video camera screening security system with connections to veterinary services, pathology, and CRES administration offices will provide improved security and efficiency, as well as better serve our visitors - $5,000.
- ★ **Muffle furnace.** The analytical chemistry division needs this equipment to clean its instruments - $2,336.
- ★ **PCR cycler.** Needed to cut down experiment time in DNA research: from a potential 12-day experiment, the cycler takes only one day to provide results - $9,435.
- ★ **Osmometer.** This equipment is used to measure pressure changes in animal fluids - $5,000.
- ★ **Orbital shaking platform.** Needed in DNA research to replace old and unreliable equipment - $2,000.
- ★ **DNA fluorometer.** Two fluorometers are needed: one in infectious diseases division and the other for molecular genetics. Both will replace an old piece of equipment and will be used in various genetic studies - $3,800 each.
- ★ **Mobile molecular laboratory (portable PCR minicycler).** This item is requested by both the immunology/virology and molecular genetics divisions. The apparatus is an entire laboratory for DNA analysis that can be packed into a single suitcase and used in field work - $3,820 each.
- ★ **Power Macintosh computer.** The ecology division needs this computer for use by a postdoctoral fellow - $3,000.

CRES REPORT
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

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SAN DIEGO WILD ANIMAL PARK

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